

<120> Recombination systems and methods for eliminating nucleic acid sequences from genome of eukaryotic organisms 53262-20031.00/13173-00010-US <130> <140> US 10/750,891 <141> 2004-01-05 <150> PCT/EP02/07281 <151> 2002-07-02 DE 101 31 786.7 <150> 2001-07-04 <151> <160> 30 <170> PatentIn version 3.3 <210> 1 <211> 788 <212> DNA <213> Saccharomyces cerevisiae <220> <221> CDS <222> (62)..(766) <223> open reading frame coding for I-SceI <400> 1 qqatccagta ctgtacctag aatacaaaga agaggaagaa gaaacctcta cagaagaagt 60 q atq aaa aac atc aaa aaa aac cag gta atg aac ctg ggt ccg aac tct 109 Met Lys Asn Ile Lys Lys Asn Gln Val Met Asn Leu Gly Pro Asn Ser 1 aaa ctg ctg aaa gaa tac aaa tcc cag ctg atc gaa ctg aac atc gaa 157 Lys Leu Leu Lys Glu Tyr Lys Ser Gln Leu Ile Glu Leu Asn Ile Glu 25 205 cag ttc gaa gca ggt atc ggt ctg atc ctg ggt gat gct tac atc cgt Gln Phe Glu Ala Gly Ile Gly Leu Ile Leu Gly Asp Ala Tyr Ile Arg 253 tct cgt gat gaa ggt aaa acc tac tgt atg cag ttc gag tgg aaa aac Ser Arg Asp Glu Gly Lys Thr Tyr Cys Met Gln Phe Glu Trp Lys Asn aaa gca tac atg gac cac gta tgt ctg ctg tac gat cag tgg gta ctg 301 Lys Ala Tyr Met Asp His Val Cys Leu Leu Tyr Asp Gln Trp Val Leu 349 tcc ccg ccg cac aaa aaa gaa cgt gtt aac cac ctg ggt aac ctg gta Ser Pro Pro His Lys Lys Glu Arg Val Asn His Leu Gly Asn Leu Val 90 85 atc acc tgg ggc gcc cag act ttc aaa cac caa gct ttc aac aaa ctg 397 Ile Thr Trp Gly Ala Gln Thr Phe Lys His Gln Ala Phe Asn Lys Leu 100 105 110 gct age ctg ttc atc gtt aac aac aaa aaa acc atc ccg aac aac ctg 445 Ala Ser Leu Phe Ile Val Asn Asn Lys Lys Thr Ile Pro Asn Asn Leu

120

125

115

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Val Glu Asn Tyr Leu Thr Pro Met Ser Leu Ala Tyr Trp Phe Met Asp
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                                            140
gat ggt ggt aaa tgg gat tac aac aaa aac tct acc aac aaa tcg atc
                                                                   541
Asp Gly Gly Lys Trp Asp Tyr Asn Lys Asn Ser Thr Asn Lys Ser Ile
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                                        155
                                                                   589
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Val Leu Asn Thr Gln Ser Phe Thr Phe Glu Glu Val Glu Tyr Leu Val
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                165
aag ggt ctg cgt aac aaa ttc caa ctg aac tgt tac cta aaa atc aac
                                                                   637
Lys Gly Leu Arg Asn Lys Phe Gln Leu Asn Cys Tyr Leu Lys Ile Asn
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                                                     190
           180
                                                                   685
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Lys Asn Lys Pro Ile Ile Tyr Ile Asp Ser Met Ser Tyr Leu Ile Phe
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tac aac ctg atc aaa ccg tac ctg atc ccg cag atg atg tac aaa ctg
Tyr Asn Leu Ile Lys Pro Tyr Leu Ile Pro Gln Met Met Tyr Lys Leu
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                                            220
ccq aac act atc tcc tcc qaa act ttc ctg aaa taataagtcg agtactggat 786
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Ile Thr Trp Gly Ala Gln Thr Phe Lys His Gln Ala Phe Asn Lys Leu
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Ala Ser Leu Phe Ile Val Asn Asn Lys Lys Thr Ile Pro Asn Asn Leu
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Val Leu Asn Thr Gln Ser Phe Thr Phe Glu Glu Val Glu Tyr Leu Val
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Lys Gly Leu Arg Asn Lys Phe Gln Leu Asn Cys Tyr Leu Lys Ile Asn
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           180
                                                     190
Lys Asn Lys Pro Ile Ile Tyr Ile Asp Ser Met Ser Tyr Leu Ile Phe
                            200
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Gly Pro Lys Lys Arg Lys Val Ile Met Ser Leu Thr Gln Gln
aaa qac tta att ttc qqa tct cta ctg ggt gat gga aat tta caa act
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Lys Asp Leu Ile Phe Gly Ser Leu Leu Gly Asp Gly Asn Leu Gln Thr
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                                                                   200
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cat cag aca tac tta ttt cat aag tat gaa atc tta aag ccg ctt tgt
                                                                   248
His Gln Thr Tyr Leu Phe His Lys Tyr Glu Ile Leu Lys Pro Leu Cys
                     55
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Gly Glu Asn Thr Leu Pro Thr Glu Ser Ile Val Phe Asp Glu Arg Thr
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                                     75
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Asn Lys Glu Val Lys Arg Trp Phe Phe Asn Thr Leu Thr Asn Pro Ser
                                                                   392
tta aaa ttc ttc gca gac atg ttc tac aca tat gac caa aac aca caa
Leu Lys Phe Phe Ala Asp Met Phe Tyr Thr Tyr Asp Gln Asn Thr Gln
                            105
                                                 110
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Lys Trp Val Lys Asp Val Pro Val Lys Val Gln Thr Phe Leu Thr Pro
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                                             125
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                                                                   488
Gln Ala Leu Ala Tyr Phe Tyr Ile Asp Asp Gly Ala Leu Lys Trp Leu
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                                        140
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                150
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Gly Thr Ile Arg Ile Gln Lys Ala Leu Lys Thr Leu Tyr Asn Ile Asp
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Thr Thr Leu Thr Lys Lys Thr Leu Gln Asp Gly Arg Ile Gly Tyr Arg
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ata gct att cct gaa gcc agt agc ggt gct ttt cgt gaa gtc att aaa
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Ile Ala Ile Pro Glu Ala Ser Ser Gly Ala Phe Arg Glu Val Ile Lys
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cct ttt cta gtt gat tgt atg aga tac aaa gtt tct gat ggc aat aaa
                                                                   728
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mu tali a ali

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Asp Thr Thr Leu Thr Lys Lys Thr Leu Gln Asp Gly Arg Ile Gly Tyr
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Arg Ile Ala Ile Pro Glu Ala Ser Ser Gly Ala Phe Arg Glu Val Ile
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Gly Pro Lys	5			10					15			
gag ttc tta Glu Phe Le	a ctc tac	tta gca	ggg	ttt	gta	gac	ggt	gac	ggt	agc	ata	153
20)		25					30				201
atc gct caa Ile Ala Gli												201
35	Tite bys	40	0111	501	- y -	Lyo	45	2,0		01	200	
tca ctc gc												249
Ser Leu Ala	a Phe Gln	Val Thr 55	Gln	Lys	Thr	Gln 60	Arg	Arg	Trp	Phe	Leu 65	
gac aaa tta	a gtg gat	gaa att	ggg	gtt	ggt	tat	gta	aga	gat	agg	ggt	297
Asp Lys Let	ı Val Asp	Glu Ile	Gly	Val	Gly	Tyr	Val	Arg	Asp	Arg	Gly	
	70			~~~	75	224	aat	++~	ast	80	+++	345
agc gtt tco Ser Val Se												242
Ser var se.	85	TIC BCu	JCI	90	110	170	110	Lou	95		1110	
tta aca caa												393
Leu Thr Gli		Pro Phe		Lys	Leu	Lys	Gln		Gln	Ala	Asn	
tta gtt tta		n++	105	at t	cca	tca	aca	110	722	tcc	cca	441
Leu Val Le	ı aaa all ı Lvs Ile	Tle Glu	Gln	Leu	Pro	Ser	Ala	Lvs	Glu	Ser	Pro	331
115	2 270 110	120					125	-1-				
gac aaa tt												489
Asp Lys Phe	e Leu Glu	_	Thr	Trp	Val		Gln	Ile	Ala	Ala		
130 aat gat to		135	202	act	tct	140	200	att	cat	act	145	537
Asn Asp Se												
-	150				155					160		
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1	5 	M T	70.1	C1	10	Wa l	7 00	C1	Λαν	15	Cor	
Lys Glu Ph	е њеш њеш 20	туг цец	Ата	25	Pne	vaı	Asp	GIY	30	GLY	261	
Ile Ile Al	a Gln Ile	Lys Pro	Asn	Gln	Ser	Tyr	Lys	Phe	Lys	His	Gln	
3			40					45				
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Leu Asp Ly	s Leu Val		Ile	Gly	Val		Tyr	Val	Arg	Asp		
65 Gly Ser Va	l Ser Asn	70 Tvr Tle	Ţ.e.ii	Ser	Glu	75 Tle	Lvs	Pro	Leu	His	80 Asn	
ory bor va	85				90		_, •			95		
Phe Leu Th		Gln Pro	Phe		Lys	Leu	Lys	Gln		Gln	Ala	
7 am 7 am 7.	100	T] ~ T] -	C1	105	T 0	D∽∽	C~~	71 a	110	C1	80~	
Asn Leu Va	r ren rys	TIG IIG	GIU	GIN	ьeu	LT.0	ser	WIG	ոչ	GIU	Set	

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115
                            120
Pro Asp Lys Phe Leu Glu Val Cys Thr Trp Val Asp Gln Ile Ala Ala
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Pro Lys Lys Lys Arg Lys Val Ile Met Asp Ile Asn Pro Gln Trp Ile
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                                                                   153
Thr Gly Phe Val Asp Gly Glu Gly Cys Phe Ser Val Ser Ile Leu Arg
                         25
aat aat tcg ttg cgc tat ggc cat cag ctt caa cca gaa ttc gta gtg
                                                                   201
Asn Asn Ser Leu Arg Tyr Gly His Gln Leu Gln Pro Glu Phe Val Val
acc caa cat aaa tta gat gca aat gtt tta tat gca tta aaa gac tac
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Thr Gln His Lys Leu Asp Ala Asn Val Leu Tyr Ala Leu Lys Asp Tyr
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                                     60
ttt aaa gtt gga tca gtc gtt gtg aat cat ggg gaa cgg ctt tgc tat
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Phe Lys Val Gly Ser Val Val Val Asn His Gly Glu Arg Leu Cys Tyr
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                                 75
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                                                                   345
Lys Val Lys Asn Ile Asp His Phe Ile Thr Val Ile Ile Pro Phe Phe
                             90
qaa aaa cat gag cta aaa aca aaa aga aga att gaa ttt ctt cga ttt
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Glu Lys His Glu Leu Lys Thr Lys Arg Arg Ile Glu Phe Leu Arg Phe
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    100
cga aaa atc tgc ttg ctg tta aaa gca ggt aga cat tta gaa tcg cag
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Arg Lys Ile Cys Leu Leu Lys Ala Gly Arg His Leu Glu Ser Gln
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                                         125
                                                                   489
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gag aaa aac tac cag gaa tct atc aaa cgt ttt gaa gaa act ggc gag
                                                                   537
Glu Lys Asn Tyr Gln Glu Ser Ile Lys Arg Phe Glu Glu Thr Gly Glu
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                                155
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Asp Tyr Phe Lys Val Gly Ser Val Val Val Asn His Gly Glu Arg Leu
Cys Tyr Lys Val Lys Asn Ile Asp His Phe Ile Thr Val Ile Ile Pro
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Pro Lys Lys Arg Lys Val Ile Met Thr Asp Ser Lys Ser Arg Asn
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                                                                   154
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Asn Asn Asn Phe Leu Ser Asn Asn Leu Leu Pro Leu Thr Asp Asp Glu
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                         25
aag gct tta att gcg ggg aca ctt tta ggg gat gct cat att caa aag
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Lys Ala Leu Ile Ala Gly Thr Leu Leu Gly Asp Ala His Ile Gln Lys
                     40
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ttg gtc tta gca g Leu Val Leu Ala A 150						
tgt tat tca gga a Cys Tyr Ser Gly I 165						
agc cag ttg ttg t Ser Gln Leu Leu C 180						
gta gtt gct cat a Val Val Ala His I 195						
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Asp Glu Lys Ala L 35	eu Ile Ala	Gly Thr	Leu Leu	Gly Asp 45	Ala His	Ile
Gln Lys Arg Gly A	sp Ser Tyr. 55	Arg Leu	Lys Ile	Ala His 60	Gly Leu	Asp
His Glu Glu Leu V	al Val Trp 70	Lys Tyr	Asn Arg 75	Leu Ile	Arg Leu	Cys 80
Gln Thr Thr Gln F	ro Pro Arg 85	Val Glu	Thr Tyr 90	Ser Thr	Lys Leu 95	Lys
Ser Gly Val Leu F 100	ro Gln Gly	Val Val 105	Phe Tyr	Thr Ser	Ser Gly 110	Lys

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Tyr Leu Lys Glu Thr Tyr Asp Leu Phe Tyr Lys Gln Thr Ala Asp Gly
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        115
Arg Arg Val Lys Thr Ile Thr Gln Glu Leu Ile Asp Ser Leu Pro Lys
                        135
His Pro Leu Val Leu Ala Ala Phe Phe Met Asp Asp Gly Ser Val Arg
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                    150
Ser Asp Cys Tyr Ser Gly Lys Ile Ala Thr Pro Gly Phe Ala Gly Lys
                                    170
                165
Glu Glu Ser Gln Leu Leu Cys Asn Tyr Leu His Ser Trp Asp Val Gln
                                185
Ala Asn Val Val Ala His Lys Lys Ala Asn Asn Gln Tyr Tyr Ile Gly
                            200
                                                 205
Leu Pro Ala Lys Thr Phe Gly Arg Phe Ile Asn Ile Ile Glu Pro Tyr
                        215
                                             220
Val Arg Glu Val Pro Ala Leu Cys Tyr Lys Leu Asn Glu Ser Arg Lys
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ggtccaaaga aaaagagaaa ggttatcatg gacattaatc ctcaatggat tacagg
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<223> Description of the artificial sequence:
      oligonucleotide primer
<400> 24
                                                                    34
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<210> 25
<211> 62
<212> DNA
<213> Artificial Sequence
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<223> Description of the artificial sequence:
      oligonucleotide primer
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tc
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      oligonucleotide primer
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ta
<210> 27
<211> 75
<212> DNA
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<213> Artificial Sequence

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<220>
<223> Description of the artificial sequence:
      oligonucleotide primer
<400> 27
ctagtccgaa aacgccgtga gacatattgg ttacgatcct aaggtagcga aattcacccg 60
gtaactctgt gccag
<210> 28
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cacggcgttt tcgga
<210> 29
<211> 7
<212> PRT
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<223> Description of the artificial sequence: nuclear
     location sequence
<400> 29
Pro Lys Thr Lys Arg Lys Val
                  5
<210> 30
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<212> PRT
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     location sequence
<400> 30
Pro Lys Lys Lys Arg Lys Val
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